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Basal cAMP/Pka/Ca^{2+} Signaling is Linked to Action Potential (AP) Rhythmicity of Sinoatrial Nodal Cells (SANC) as well as to their Firing Rate

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Compared to freshly isolated SANC (f-SANC), the spontaneous AP firing rate at 34 ± 0.5°C of cultured adult rabbit SANC (c-SANC) is reduced by 50% (from 2.79Hz to 1.35Hz), due to G_i protein suppression of basal cAMP/PKA/Ca^{2+}-dependent signaling. Here we demonstrate that altered PKA-dependent modulation of basal intracellular Ca^{2+} cycling also reduces AP rhythmicity of c-SANC.

The AP rhythmicity index (RI, fig.1A), i.e. the offset of the 3rd peak from the autocorrelation function of AP records, or from power spectrum analysis is reduced in c- vs. f-SANC, and is associated with prolongation of spontaneous Local Ca^{2+} Releases (LCR) period during diastolic depolarization and an increase in its coefficient of variation (0.199 ± 0.014 (n=41) for c-SANC vs. 0.122 ± 0.009 (n=32) for f-SANC, $p<0.001$). Acute β-adrenergic receptor stimulation by isoproterenol (ISO), phosphodiesterase inhibition by 3-isobutyl-1-methylxanthine (IBMX), or prolonged G_i suppression by pertussis toxin (PTX), which rescues impaired cAMP/PKA signaling in c-SANC, not only rescues the reduced AP firing rate, but also restores normal variability of LCR period and restores the rhythmicity of AP firing to the f-SANC level (fig.1B).

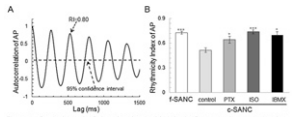

*Figure 1. Rhythmicity of AP in c-SANC and f-SANC. A. Representative example of an autocorrelation function of AP recording. RI is indicated. B. Average data of APs RI in f-SANC and c-SANC (at control or with intervention) (n=9-74 for each group, *, p<0.05 or ***, p<0.001 compared to c-SANC control via one-way ANOVA)*

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Regional Variations of the Effects of Acetylcholine in the Canine Heart

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Acetylcholine (ACh) release slows heart rate and atrioventricular conduction by stimulation of an inward rectifying current (IK,ACh) in atrial tissue. The effect of ACh on ventricular function is still debated. We compared the effect of ACh on APs in canine atria, Purkinje and ventricular tissue as well as on ionic currents in isolated cells. Action potentials were recorded from endo- or epicardial slices, Purkinje fibers, or atrial preparations. Whole-cell currents were recorded under voltage clamp conditions and unloaded cell shortening determined by video edge detection. The effects of ACh (1-10 μM) on IK,ACh and I_{Ca} in the 4 cell types were measured. In atrial tissue, application of ACh hyperpolarized the membrane potential and shortened action potential duration (APD). In Purkinje and ventricular tissues, no significant effect of ACh on APD, membrane potential or dV/dt was observed at 1 Hz pacing. Under voltage clamp, addition of ACh to atrial cells activated a large inward rectifying current (from −3.5 ± 0.7 to −23.7 ± 4.7 pA/pF) that was abolished by tertiapin, a specific blocker of IK,ACh. No significant enhancement of this current was observed in the other cell types following ACh application. A small inhibition of I_{Ca} was observed in all cell types after ACh. This I_{Ca} inhibition became greater at fast pacing rates. In the canine heart, application of ACh resulted in a marked reduction in APD in atrial tissue only. The effect on APD could be inhibited by an IK,ACh blocker suggesting this channel type was predominantly expressed in atrial tissue. In epi, endo and Purkinje tissue, no significant effect of ACh on APD was observed. A minor rate-dependent effect of ACh on I_{Ca} was noted however, the effect of this finding may be negligible at physiologically relevant rates.

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Effects of SK Channel Blockers on Atrial Myocytes Suggest SK Channel Heterogeneity

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Atrial fibrillation (AF) is the most common sustained arrhythmia and contributes to cardiac morbidity and mortality. Extension of the atrial effective refractory period through block of potassium channels is a therapeutic strategy for AF, with an atrial-selective drug being desired to avoid potentially lethal ventricular arrhythmias. Small-conductance calcium-activated potassium (SK) channels have been recently suggested as a promising atrial selective target, but disagreement exists concerning expression and function of these channels in atrial myocytes. Visualization of antibody labelling using confocal microscopy showed the presence of SK2 protein in mouse atrial myocytes with localization of staining along the z-lines. Whole-cell recordings revealed outward currents positive to −20 mV that were sensitive to two SK inhibitors, apamin and UCL1684. Current was blocked by apamin with an IC_{50} of 118 pM, close to reported values for homomeric SK2 current in mammalian cell lines. Action potential duration (APD) was prolonged more by application of UCL1684 than apamin, at a firing frequency of 0.2 Hz. The effect of UCL1684 was greater with a firing frequency of 2 Hz, producing a decrease of the stability of APD and increasing beat-to-beat variability (BVR) in APD. These data suggest that functional SK channels are present in the mouse atrium. However, the effects of apamin were different under voltage- or current-clamp conditions, while the effects of UCL1684 were similar. This difference might arise from atrial myocytes expressing more than one population of SK channels, with one being apamin-insensitive and contributing to action potential repolarization. The effect of UCL1684 on APD is consistent with the recruitment of more SK channel activity at higher firing frequencies. Block of these SK channels increases BVR, a marker of drug induced repolarization-related proarrhythmias, raising the possibility that SK inhibition could be proarrhythmic.

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Mechanisms of Steeper APD Restitution in Rat Failing Right Ventricular Myocytes

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Pulmonary artery hypertension (PAH) causes the right ventricle (RV) to become hypertrophied and fail. During this process the RV undergoes electrophysiological remodeling with resultant changes in action potential duration (APD). This study evaluated changes in APD restitution in a rodent model of PAH.

Male Wistar rats were given a single i.p. injection of monocrotaline (60 mg/kg) or an equivalent volume of saline. When clinical symptoms of heart failure became apparent (3-4 weeks later) animals were euthanized and the hearts excised. RV myocytes were enzymatically isolated and used for a number of electrophysiological measurements. These included: measurements of APD at pacing rates between 1 and 9 Hz; an action potential (AP) clamp to impose the AP recorded at 1 Hz at pacing rates of 1, 2, 5 and 7 Hz; and measurements of APD during application of increased negative current pulses between 1 and 500 pA in amplitude at 1 and 5 Hz.

Myocytes from the failing right ventricle had a significantly longer APD and steeper APD steeper restitution curve compared to sham cells. Despite this greater APD shortening, under action potential clamp, compensation currents in failing myocytes were smaller in amplitude as pacing frequency increased. Consistent with this observation, injection of negative current caused a greater decrease in APD_{90} in failing cells ($P < 0.0001$-0.05, n = 6-7 myocytes, ANOVA, Tukeys post-test).

These findings show that cells isolated from the monocrotaline treated RV required less current to cause a decrease in APD, which is consistent with an increased membrane resistance. Previous studies have reported a decreased expression of potassium channels in monocrotaline-treated myocytes, which may be a contributing factor responsible for the results observed.

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Changes of Axial Resistance following Mechanical Strain Prevail Over Stretch-Activated Currents in the Modulation of Conduction Velocity in Cardiac Cell Strands

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Tissue deformation and stretch-activated currents (I_{SAC}) exert a feedback on cardiac electrical function (mechano-electrical feedback). The effects of stretch on conduction velocity (CV) and their modulation by I_{SAC} are still debated. We investigated the dependence of CV on passive tissue deformation and its modulation by I_{SAC} in cultured cardiomyocyte strands and simulation studies.

Strands of neonatal rat ventricular myocytes were cultured on deformable substrates. CV was measured optically under control conditions, upon 10% shortening and 10% lengthening. Simulations were conducted in fibers of ten Tusscher et al. model cells. A quadratic dependence of myoplasmic resistance on cell length was incorporated and gap junctional resistance (equal to myoplasmic resistance under control conditions) was assumed to be unaffected. I_{SAC} was implemented as a constitutively active non-specific monovalent cation current with a nonlinear dependence on deformation.

In cultured strands, CV decreased by 3.0% upon 10% shortening and increased by 3.9% upon 10% lengthening (n=25). In simulated fibers without I_{SAC}, CV decreased by 5.1% and increased by 4.2% upon 10% shortening and 10% lengthening, respectively, in agreement with the experiments. When I_{SAC} was incorporated at previously reported levels, it caused a slight resting membrane depolarization by ~1 mV in undeformed fibers, but no major alteration of the CV behavior (4.8% decrease at 10% shortening; 3.9% increase at 10%

lengthening). At large I_{SAC} levels causing substantial membrane depolarization ($\geq$5 mV) and inactivation of the Na^+ current, the dependence of CV on tissue deformation was blunted or even inverted, with lengthening causing conduction slowing.

Thus, during length changes of $\pm$10%, axial tissue resistance and I_{SAC} modulate conduction in opposite directions. However, at physiological I_{SAC} levels, CV is primarily determined by axial tissue resistance.

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Resolution of Hypo-Osmotic Stress in Isolated Mouse Ventricular Myocytes Leads to Detubulation

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It has been recently shown that various stress-inducing manipulations in isolated ventricular cardiac myocytes may lead to significant remodeling of t-tubules. Osmotic stress is one of the most common complications in various experimental and clinical settings, and therefore, this study was designed to test a hypothesis that osmotic challenge may affect the integrity of t-tubules. T-tubular remodeling in mouse ventricular myocytes in response to various osmotic challenges was studied using two approaches: (1) electrophysiologically, by measuring membrane capacitance and I_{K1} tail currents originating from K^+ accumulation in ttubules, and (2) using confocal microscopy of fluorescent dextrans trapped in vesiculated t-tubules. In particular, application and removal of 0.6 T (60% of NaCl) hypo-osmotic solution to myocytes led to ~30% reduction in membrane capacitance, ~3-fold reduction in the amplitude of I_{K1} tail current and ~2-fold reduction in so-called I_{K1} 'inactivation' (due to depletion of t-tubular K^+) at negative membrane potentials – all being consistent with strong detubulation. Importantly, confocal imaging experiments showed that extracellularly applied dextrans become trapped inside the myocytes only upon removal of hypo-osmotic solutions (i.e. during shrinking phase) but not during initial swelling period. In light of these data some relevant previous studies, including those on EC coupling phenomena during hypo-osmotic stress, may need to be reinterpreted and the experimental design of future experiments should take into account the novel findings.

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Fluid Pressure-Activated Non-Selective Cation Current and Cl^- Current in Rat Atrial Myocytes

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When intact atrial muscle is exposed to turbulant flow or high fluid pressure during valve diseases, it produces arrhythmias. Here we characterized a novel fluid pressure (FP)-gated ionic current (I_{FP}) in single rat atrial myocytes using a whole-cell patch clamp. A flow of pressurized (~16 dyn/cm^2) fluid was applied onto single rat atrial myocytes using a microperfusion method. The application of FP with a normal bath solution elicited a transient inward current (~1 pA/pF at $-$80 mV). The magnitude of I_{FP} was increased in a pressure-dependent manner. The removal of extracellular Ca^{2+} largely enhanced the I_{FP} and eliminated the current adaptation. Under physiological ionic gradients, the I_{FP} displayed an inwardly- and outwardly-rectifying current-voltage relationship with a reversal potential (E_{rev}) of approximately $-$52 mV. The Cl^- channel blockers, DIDS and 9-AC, suppressed inward and outward I_{FP} by about 50% and 70-80%, respectively. In symmetrical Cl^- solutions, the E_{rev} was shifted rightward ($\cong -18$ mV) and the outwardly rectifying I_{FP} was attenuated. In the symmetrical Cl^- conditions, removal of extracellular Na^+ largely reduced inward I_{FP}, and produced a left shift of E_{rev} ($\cong -64$ mV). In addition, the elimination of internal K^+ shifted E_{rev} to $\cong +8.4$ mV and decreased outward I_{FP}. Although low concentrations of extracellular Ca^{2+} blocked I_{FP} with a negative shift of E_{rev}, high concentrations of extracellular Ca^{2+} produced a right shift of E_{rev}. Gadolinium ion (Gd^{3+}), the stretch-activated channel blocker, partially blocked the inward I_{FP}. In current-clamped cells, FP of the same magnitude elicited spontaneous membrane depolarization with repetitive action potentials and prolonged action potential durations. These results indicate that FP may activate an outwardly rectifying Cl^- channel and a Gd^{3+}- and Ca^{2+}-sensitive non-selective cation channel that carries Na^+, K^+, and Ca^{2+}.

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Cardiac Na/K-ATPase and Na/Ca Exchange Function is Altered in Ankyrin B Heterozygous Mice

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Ankyrin-B (AnkB) is a multivalent ''adaptor'' protein that targets select membrane proteins to the cytoskeleton. Loss-of-function mutations in AnkB may cause ventricular arrhythmias and sudden cardiac death in humans. Direct interaction with AnkB is required for the membrane targeting and stability of Na/Ca exchanger (NCX) and Na/K-ATPase (NKA), key regulators of cardiac contractility and arrhythmogenesis. However, it is currently unknown how AnkB modulates NCX and NKA function. To investigate this, we used AnkB heterozygous mice ($AnkB^{+/-}$) and their wild-type (WT) littermates. Cardiac myocytes from $AnkB^{+/-}$ mice show reduced expression (by ~20%) and altered localization of both NCX and NKA. In agreement with the lower protein level, we found slower decay of the caffeine-induced Ca transient (τ=7.4$\pm$0.8 sec *vs*. 5.2$\pm$0.6 sec) and reduced maximum rate of NKA-mediated Na extrusion (5.0$\pm$0.5 *vs*. 6.4$\pm$0.4 mM/min) in intact myocytes from $AnkB^{+/-}$ mice *vs*. WT. Thus, NCX and NKA transport function are reduced in $AnkB^{+/-}$*vs*. WT mice. We also measured the voltage-dependence of the currents carried by NCX (I_{NCX}) and NKA (I_{pump}) using whole-cell voltage-clamp. I_{NCX} and I_{pump} were recorded during descending voltage ramps, as Cd-sensitive and K-activated currents, respectively. I_{NCX} reflected the lower NCX expression in $AnkB^{+/-}$ myocytes, with no difference in the voltage-dependence *vs*. WT. In contrast, I_{pump} had a significantly ($p<0.001$) steeper voltage-dependence in $AnkB^{+/-}$*vs*. WT myocytes. Thus, at $-$80 mV, close to the resting membrane potential, I_{pump} was reduced by ~35% in $AnkB^{+/-}$ mice, whereas at +30 mV, close to the peak of the action potential, $AnkB^{+/-}$ I_{pump} was elevated by ~18% *vs*. WT. Thus, in addition to reducing NKA protein expression, AnkB also directly modulates NKA function in cardiac myocytes, by reducing the voltage-dependent I_{pump} inactivation. This could significantly affect myocyte $[Na]_i$ and $[Ca]_i$ regulation.

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Electrophysiologic Effects of Azithromycin in Cardiomyocytes

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The widely-used macrolide antibiotic azithromycin (AZ) increases risk of cardiovascular and sudden cardiac death. Case reports indicate that AZ can cause polymorphic ventricular tachycardia in the absence and presence of QT prolongation, implying a novel proarrhythmic syndrome. We investigated the electrophysiologic effects of AZ *in vivo* and *in vitro* using mice, cardiomyocytes, and heterologously-expressed human ion channels. After implanting an ECG telemeter, conscious adult mice received intraperitoneal injection of AZ (50 mg/kg, followed in 60 min by 100 mg/kg; n=7). With both doses of AZ, heart rate declined (from 685$\pm$24 to 489$\pm$20 and 481$\pm$21 bpm, for baseline, 50 and 100 mg/kg, respectively [mean$\pm$SEM]; $P<0.001$). In addition, AZ increased the PR interval (32.7$\pm$0.9 ms to 39.4$\pm$0.7 and 39.8$\pm$0.9 ms, respectively; $P<0.001$), QRS interval (10.2$\pm$0.4 ms to 12.5$\pm$0.4 and 13.3$\pm$0.5 ms, respectively; $P<0.001$), and QT interval (37.4$\pm$4 ms to 48.0$\pm$5 and 51.2$\pm$4 ms, respectively; $P<0.01$). In spontaneously-beating HL-1 cardiomyocytes, AZ (100 μM) significantly slowed beat rate (from 215$\pm$7 to 180$\pm$7 bpm; n=14; $P<0.01$), while increasing action potential rise time (23.0$\pm$3.0 to 36.2$\pm$3.8 ms; $P<0.01$) and duration (at 90% repolarization, 118.3$\pm$8 to 137.8$\pm$8.7ms; $P<0.01$). In HEK cells stably expressing SCN5A, AZ reduced Na^+ currents (IC_{50} 110$\pm$3 μM; n=14), while similar results were obtained using mouse ventricular myocytes (IC_{50} 117$\pm$4 μM; n=6). In addition, AZ suppressed K^+ currents recorded from HEK cells expressing hERG (IC_{50} 219$\pm$21 μM; n=5) and CHO cells expressing KCNQ1 and KCNE1 (IC_{50} 184$\pm$12 μM; n=6), as well as L-type Ca^{++} current in rabbit ventricular myocytes (IC_{50} 67$\pm$4 μM; n=5). We conclude that azithromycin blocks multiple cardiac ion channels to prolong the PR, QRS, and QT interval *in vivo*, at concentrations achievable within the heart based on intracellular drug accumulation. These effects likely contribute to its novel proarrhythmic effect in humans.

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Enhancement of Antioxidant Defence Preserves RyR2 Function of Hyperglycemic Cardiomyocytes via Regulation of both Intracellular Zn^{2+} and Ca^{2+} Homeostasis

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Zinc exists in biological system and resting intracellular level of free Zn^{2+} ($[Zn^{2+}]_i$) can be greatly increased by thiol-reactive oxidants or high glucose and contributes to oxidant-induced alterations in EC-coupling although in cardiomyocytes. Since $[Zn^{2+}]_i$ is altering function of numerous cellular proteins, its mobilization by reactive oxygen species in diabetic heart can be likely to cause significant effects. Therefore, we aimed to investigate the role of antioxidant-defence system in preserving of cardiac ryanodine receptor